## Validation of an automated, enzymatic, human assay for determination of serum and plasma lithium Image: I

Laminitis is a common condition in horses that can develop from derangements such as metabolic stresses from a high carbohydrate diet, ingesting rich spring grass or toxins (e.g. black walnut). Laminitis is an inflammatory condition of the hoof whereby the epidermal and dermal lamina separate. In severe cases, the complete destruction of this dermalepidermal interface allows for the rotation of P3 through the sole of the hoof leading to imminent euthanasia of the animal. Because of the high incidence in horses and the potential devastating effects that can occur, a possible treatment for laminitis has emerged as a topic of interest in veterinary research. More specifically, lithium chloride has shown antiinflammatory effects and may be useful for ameliorating disease progression. Although it is used therapeutically in human medicine, the effects in veterinary medicine are unknown. In order to advance research on the use of lithium as a therapeutic agent in horses, a precise and validated blood assay to detect plasma levels of lithium is necessary.



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## **Materials and Methods**

All samples were analyzed using an human enzymatic lithium assay (Diazyme Labs, Poway, CA) on an automated clinical chemistry analzyer (Beckman-Coulter, Inc., Bray, CA). Serum and EDTA plasma used for assay validation was obtained from two horses administered a bolus (60mM) of lithium chloride (LiCl) via jugular vein and samples drawn at specific time points over a 24h period. For assay validation, several bioanalytical methods were used. Assay linearity was assessed via two different protocols: 1) a pre-dose serum samples spiked with a known concentration of LiCl and serially diluted and 2) serially diluting pooled high serum with pooled low serum. An interday coefficient of variation was determined, as well as, spiked sample recovery and sample stability after 24h of refrigeration and a freeze/thaw cycle. Finally all plasma and serum samples taken from the experimental horses were analyzed to determine the pharmacokinetic pattern.

## Results

The assay displayed relative linearity using both dilution protocols. (Figures 1 and 2) Plasma and serum lithium concentrations in the two horses given a bolus of LiCL displayed an expected pharmacokinetic pattern. (Figure 3) Because the varying serum and plasma concentrations were similar, it was determined the either would be an acceptable samples for the Lithium assay. The assay showed acceptable precision with an interday CV of 2.5% and recovery of a lithium from a spiked serum sample was 96.3%. After 24h of refrigeration and 24h freeze/thaw cycle showed similar lithium concentrations to fresh plasma and serum samples. (Figures 4 and 5)



Figure 2 – Linearity of assay using pooled high and pooled low serum.





Figure 5 – Stability of plasma and serum samples after a 24h freeze/thaw cycle.

## Conclusions

In this study, a human lithium assay appears to be valid for use in precisely measuring Li in equine plasma and serum. Based off the averaged table of serum versus plasma, either one can be used to determine blood levels of lithium. Furthermore, results show no change after freezing or refrigerating for 24hrs post draw. This outcome can benefit future studies in determining therapeutic levels of Li necessary to prevent or cure laminitis in equines. Future validation shall include correlation of plasma and serum lithium concentrations to a gold standard. Acknowledgments A sincere thank you to everyone who helped with this projectespecially my mentors Dr. Wiedmeyer and Dr. Johnson. A genuine thank you to the financial supporters, the Animal Health Foundation of St Louis and to Bayer Animal Health, who allowed this study to be more than idea to set the foundation for further studies in treatment of laminitis.